

The influence of mayonnaise pH and storage temperature on the growth of *Listeria monocytogenes* in seafood salad[☆]

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Abstract

Seafood salad has been identified as a ready-to-eat food with a relatively high incidence of contamination by *Listeria monocytogenes*; however, little is known about the behavior of this pathogen in seafood salad as a function of product pH and storage temperature. To produce data towards the development of a predictive growth model, a 6-strain cocktail of *L. monocytogenes* was inoculated onto the surface of a shrimp-crabmeat product, mixed with mayonnaise that was previously adjusted with NaOH to pH 3.7, 4.0, 4.4, 4.7 or 5.1, and then stored at 4°, 8° or 12 °C under both aerobic and vacuum conditions. At each storage temperature, *L. monocytogenes* was able to grow in the seafood salad under both aerobic and vacuum conditions. The slowest growth of *L. monocytogenes* was observed in seafood salad with a mayonnaise pH of 3.7 and a storage temperature of 4 °C under vacuum condition. In salad with the same mayonnaise pH, the growth rate (GR, log₁₀ cfu/h) of *L. monocytogenes* increased as a function of storage temperature. At the same storage temperature, the lag phase duration (LPD, h) of *L. monocytogenes* decreased as mayonnaise pH increased. At the same mayonnaise pH and temperature, LPD of *L. monocytogenes* was greater under aerobic than under vacuum conditions. Regression analyses indicated that mayonnaise pH is the main effector on the LPD of *L. monocytogenes* in seafood salad, and storage temperature was the main effector on the GR. Secondary models that describe LPD and GR of *L. monocytogenes* in seafood salad as a function of mayonnaise pH and storage temperature were produced.

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Keywords: *Listeria monocytogenes*; Seafood salad; Model

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1. Introduction

Refrigerated ready-to-eat (RTE) foods are ready for consumption without additional preparation and cooking by consumers. Examples of refrigerated RTE foods are luncheon meats, deli salads, soft cheeses,

and pre-packed fresh vegetables and fruits. With its prevalence in the environment and its ability to grow at refrigerated temperatures, *Listeria monocytogenes* is a concern in refrigerated RTE foods. A recent study found that *L. monocytogenes* was present in 4.7% of seafood salads and in 2.4% of deli salads (i.e., potato salad, tuna salad, pasta salad, and coleslaw) in the United States. The levels of *L. monocytogenes* in seafood salads ranged from 0.04 to 10^3 cfu/g (Gombas et al., 2003). In Portugal, a study showed *L. monocytogenes* was present in 9.0% of 138 RTE food samples analyzed (Guerra et al., 2001). Among them, 10.5% of deli salads and 21% of RTE meats were contaminated with *L. monocytogenes*. The prevalence rate of *L. monocytogenes* in seafood salad was 16% in Iceland (Hartemink and Georgsson, 1991) and 27% in Belgium (Uyttendaele et al., 1999). McCarthy (1997) also reported that *L. monocytogenes* was recovered from 3.0% of whole boiled RTE crawfish samples in the United States.

Deli salads are normally not heat-treated before consumption; therefore, presenting a potential risk to consumers. In a risk assessment by the U.S. Food and Drug Administration/U.S. Department of Agriculture/Centers for Disease Control and Prevention (2001), deli salads, such as meat, seafood, egg, and pasta salads, had a higher predicted relative risk of causing listeriosis in the U.S. on a per serving basis, ranking second on a per annum basis among 20 RTE food categories. The assessment recommended that approaches for reducing *L. monocytogenes* contamination of deli salads should be identified.

While various scientists have investigated the behavior of *L. monocytogenes* in homogeneous environments, such as microbiological broths (Buchanan and Phillips, 1990; Buchanan and Klawitter, 1990; Nerbrink et al., 1999; LeMarc et al., 2002), as well as in a variety of formulated food products, few investigations have examined the behavior of *L. monocytogenes* in foods, such as seafood salads, that contain multiple components possessing different physical/chemical properties. Such experiments are especially relevant when no pathogen growth occurs in one food component but high levels of growth occur in another component. Consequently, pathogen growth is affected by a gradient of environmental conditions that occur around the individual components. In this study, we examined

the growth of *L. monocytogenes* in separate and mixed components of a seafood salad formulated from shrimp, imitation crabmeat, and mayonnaise stored under aerobic and vacuum conditions at refrigerated and abuse temperatures.

2. Materials and methods

2.1. Bacterial strains

A cocktail of six strains of *L. monocytogenes* obtained from the culture collection of the Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture (ERRC-ARS-USDA, Wyndmoor, PA) was used in this study. The strains were F6854 (turkey frank isolate; Schwarz et al., 1998), MFS-2 (environmental isolate from a pork processing plant; Porto et al., 2003), H7776 (frankfurter isolate; CDC, 1998), JBL2365 (chocolate milk isolate; Proctor et al., 1995), F2365 (Hispanic-style cheese isolate; Linnian et al., 1988), and 101 M (ground beef isolate; Czuprynski et al., 2002). A loopful of frozen culture of each strain was separately transferred to 10 ml of tryptic soy broth (TSB, Difco Laboratories, Detroit, MI). After a 48-h incubation at 35 °C, 0.1 ml of each individual culture was transferred to 10 ml of a 1:1 mixture (pH 4.7) of TSB and mayonnaise (Hellmann's REAL Mayonnaise; Bestfoods, Englewood Cliffs, NJ) and incubated at 35 °C for 72 h to acclimate each strain to mayonnaise components. This procedure was repeated two additional times by subculture to fresh TSB-mayonnaise mixtures. At the end of the third 72-h incubation in TSB-mayonnaise, the concentration of each strain was approximately 10^8 colony-forming-unit (cfu)/ml. Finally, a cocktail was made with 0.1 ml of each TSB-mayonnaise culture and diluted with sterile 0.1% peptone water to produce an experimental inoculum of 10^6 cfu/ml.

2.2. *L. monocytogenes* in mayonnaise

The initial pH of mayonnaise was 3.7. The pH of mayonnaise was adjusted by mixing 200 g of mayonnaise with 10% NaOH for pH 4.0 and 4.4, or 50% NaOH for pH 5.0, in 400-ml capacity stomacher

bags (Spiral Biotech, Norwood, MA) with a Bag-Mixer 400 Stomacher (Interscience, St. Nom, France). For each 200 g of mayonnaise, approximately 0.50 ml and 1.0 ml of 10% NaOH were needed to adjust mayonnaise pH to 4.0 and 4.5, respectively, and 0.75 ml of 50% NaOH for pH 5.0. The pH-adjusted mayonnaise was stored at 4 °C for 24 h. Five milliliters of the six-strain *L. monocytogenes* cocktail was inoculated into 100 g of mayonnaise to achieve a final level of $\sim 10^4$ *L. monocytogenes*/g. Next, 30 g of the inoculated mayonnaise was placed into each of two 50-ml centrifuge tubes and stored at 4 and 8 °C. *L. monocytogenes* was analyzed in samples at a 2- or 3-day time interval up to 19 days.

2.3. *L. monocytogenes* in shrimp-imitation crabmeat mixture

Individually quick-frozen cooked shrimp and imitation crabmeat (fish protein [pollock, cod and/or whiting], water, wheat starch, egg whites, sorbitol, modified food starch, sugar, wheat gluten, soybean oil, salt, potassium chloride, natural and artificial flavors, sodium tripolyphosphate, mirin wine, tetrasodium pyrophosphate, paprika, color added, hydrogenated corn oil, soy and wheat protein, autolyzed yeast, maltodextrin, corn starch, disodium inosinate and guanylate and fractionated coconut oil) were purchased from a local grocery store. Thawed shrimp and imitation crabmeat were sealed in Stomacher bags and heat treated in an autoclave at 100 °C for 10 min to inactivate background spoilage microflora. The bags containing shrimp and crabmeat were cooled under running tap water immediately after the heat-treatment. The total microbial count in heat-treated shrimp and crabmeat was less than the detection limit of 2.0 cfu/g and no endogenous *L. monocytogenes* were detected. Five ml of the six-strain *L. monocytogenes* cocktail were added to 200 g of the shrimp-imitation crabmeat mixture (100 g:100 g) in a 720-g capacity stomacher bag and mixed by hand massage for 2 min. Sixty grams of the inoculated seafood were placed into each of three 237-ml capacity sterile polypropylene containers with snap lids (Fisher Scientific, Pittsburgh, PA), and stored separately at 4, 8, and 12 °C for up to 7 days. Each sample was tested for *L. monocytogenes* levels in 2- to 3-day time intervals.

2.4. *L. monocytogenes* in seafood salad

Mayonnaise was prepared to have five different pH levels (3.7, 4.0, 4.4, 4.7 and 5.1) as described above for the *L. monocytogenes*-mayonnaise study. The pasteurized 1:1 shrimp and imitation crabmeat mixture (900 g) was inoculated with a 20-ml cocktail of the six-strain *L. monocytogenes* to achieve an inoculum of $\sim 10^2$ cfu/g as described above. The *L. monocytogenes*-inoculated shrimp-crabmeat mixture was mixed with 300 g of the pH-adjusted mayonnaise in 720-ml capacity stomacher bags by hand. A 3:1 ratio of seafood to mayonnaise is commonly used to prepare commercial seafood salad products (Anonymous, 2003). For aerobic storage, 150 g of the seafood salad were placed in 237 ml-capacity sterile polypropylene containers with snap lids. For vacuum-packaged storage, 5 g of the seafood salad were packed into 100-ml Stomacher bags and the bags vacuum-sealed to –980 mbar using a Multivac A300 vacuum sealer (Multivac, Kansas, MO). Samples were stored at 4, 8 and 12 °C for up to 21 days.

2.5. Enumeration of *L. monocytogenes* in mayonnaise, seafood, and seafood salad

Throughout the studies, levels of *L. monocytogenes* were analyzed at a 2- or 3-day time interval. For studies of *L. monocytogenes* in mayonnaise, 2 g of sample were placed in 100-ml stomacher bags, mixed with an equal weight of sterile 0.1% peptone water and stomached for 2 min. Additional dilutions, if needed, were prepared in sterile 0.1% peptone water. For shrimp-imitation crabmeat studies and aerobic seafood salad samples, 5 g of sample were mixed with an equal weight of sterile 0.1% peptone water in a 100-ml bag and stomached for 2 min. For vacuum-packaged salad samples, an equal weight of sterile 0.1% peptone was added directly and stomached. Sample dilutions were spread-plated on modified Oxford agar (MOX, Oxoid, Hampshire, England), incubated at 35 °C for 48 h, and then black colonies surrounded by black precipitation that were identical to those of *L. monocytogenes* stock culture on MOX plates were counted. If needed, colonies recovered from samples were confirmed using A PI test strips (BioMerieux, Marcy l'Etoile, France). At each sam-

Table 1

Initial pH and water activity (a_w) of shrimp, imitation crabmeat, mayonnaise, and seafood salad. Measurements were means of two samples

Component	pH ^a	a_w ^a
Shrimp	8.2	0.99
Crabmeat	7.0	0.98
Mayonnaise	3.7, 4.0, 4.4, 4.7, 5.1	0.95
Seafood salad (mayonnaise pH 3.7)	6.3	0.98
Seafood salad (mayonnaise pH 4.0)	6.5	0.98
Seafood salad (mayonnaise pH 4.4)	6.5	0.98
Seafood salad (mayonnaise pH 4.7)	6.6	0.98
Seafood salad (mayonnaise pH 5.1)	6.7	0.98

^a Means of two samples.

pling time, two samples from each treatment were analyzed.

2.6. pH and water activity measurements

The initial pH of shrimp, crabmeat, mayonnaise, and salad were measured with a Corning pH meter model 430 fitted with a Corning “3 in 1” combo electrode (Corning, New York, NY). A 1:1 portion of shrimp, imitation crabmeat, or seafood salad and deionized water were placed in a 100-ml stomacher bag and then macerated by hand for one minute before pH measurement. The pH of mayonnaise was measured directly. Water activity of samples was measured directly with an AquaLab models CX2 water activity meter (Decagon Devices, Pullman, WA). Duplicate samples were used in pH and water activity measurements.

2.7. Curve-fitting and regression analysis

The D-model described by Baranyi and Roberts (1994) was used to fit *L. monocytogenes* counts (\log_{10} cfu/g) versus time (h) to estimate lag phase duration (LPD, h) and growth rate (GR, \log_{10} cfu/h) using DMFit curve-fitting software (courtesy of Baranyi, J., Institute of Food Research, Norwich, UK). The growth rate was calculated from the linear portion of the curve. A full quadratic multiple regression analysis was used to analyze LPD and GR as functions of storage temperature and mayonnaise pH (Essential Regression and Experimental Design software; Stepan et al., 1998). Means of LPD and GR were compared with the

Tukey paired mean comparison test with significance set at 95% using a commercial software (Analyse-It Software, Leeds, England).

3. Results and discussion

3.1. Water activity (a_w) and pH of samples

The average pH and a_w of shrimp, imitation crabmeat, mayonnaise, and seafood salad are shown in Table 1. The difference in pH between shrimp (8.2) and mayonnaise was 4.5–3.1, and the difference between imitation crabmeat (7.0) and mayonnaise (3.7–5.1) was 3.3 to 1.9. The a_w of mayonnaise (0.95) was lower than shrimp (0.99) and imitation crabmeat (0.98). The pH of the seafood salad ranged from 6.3 to 6.7, with a corresponding a_w of 0.98. The pH of seafood salad (6.3) prepared with mayonnaise pH of 3.7 was comparable to a retail seafood salad (pH 6.2).

3.2. *L. monocytogenes* in seafood

L. monocytogenes grew in the 1:1 mixture of shrimp-crabmeat at storage temperatures of 4, 8, and 12 °C (Fig. 1). At an initial inoculum of approximately 2.0 \log_{10} cfu/g, the levels of *L. monocytogenes* increased to $\geq 6.0 \log_{10}$ cfu/g after 4 days at 8 and 12 °C, and after 7 days at 4 °C. The growth rates (GR \log_{10} cfu/h) of *L. monocytogenes* in seafood were 0.024 \log_{10} cfu/h at 4 °C, 0.050 \log_{10} cfu/h at 8 °C, 0.052 \log_{10} cfu/h at 12 °C.

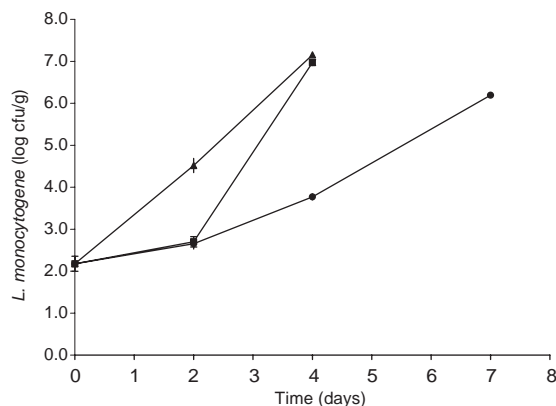


Fig. 1. Growth of *L. monocytogenes* in shrimp-imitation crabmeat mixture stored at 4 °C (●), 8 °C (■) and 12 °C (▲).

3.3. *L. monocytogenes* in mayonnaise

In mayonnaise alone, *L. monocytogenes* did not grow at pH 4.0, 4.5, and 5.0 over 19 days of storage at 4 or 8 °C (Fig. 2). *L. monocytogenes* was inactivated in mayonnaise at pH 4.0 (4.0 log₁₀ cfu/g reduction in 4 days) and 4.5 (4.0 log₁₀ cfu/g reduction in 10–15 days) at both storage temperatures, whereas no significant level of inactivation or growth was observed at pH 5.0. The inactivation rate of *L. monocytogenes* was -0.042 log₁₀ cfu/h in mayonnaise adjusted to pH 4.0 at 4 or 8 °C, -0.015 log₁₀ cfu/h in mayonnaise with pH of 4.5 at 4 °C, and -0.017 log₁₀ cfu/h at 8 °C. No significant inactivation (<-0.001 log₁₀ cfu/h) occurred in mayonnaise adjusted to pH of 5.0 at 4 or 8 °C.

3.4. *L. monocytogenes* in seafood salad

For *L. monocytogenes* inoculated onto seafood components followed by mixing with mayonnaise, growth occurred in all samples regardless of storage temperature (4, 8, and 12 °C) and mayonnaise pH (Fig. 3a–f). At an initial inoculum of approximately 1.5 log₁₀ cfu/g, the levels of *L. monocytogenes* steadily increased during storage at 4, 8 and 12 °C under aerobic conditions, regardless of the initial pH of mayonnaise. At 4 °C, *L. monocytogenes* levels in salad with mayonnaise pH 4.0–5.1 increased to 7.0 log₁₀ cfu/g after 22 days of storage. The growth in salad with

mayonnaise pH of 3.7 was the slowest and the levels were 6.0 log₁₀ cfu/g after 22 days (Fig. 3a). At 8 °C, the growth of *L. monocytogenes* was slower in salad with mayonnaise pH of 3.7 and 4.0 than those with mayonnaise pH of 4.4–5.1 within a 14-day storage, but *L. monocytogenes* levels in all samples were 7.0 log₁₀ cfu/g after 16 days (Fig. 3b). At 12 °C, although the levels of *L. monocytogenes* were lower (6.0 log₁₀ cfu/g) in salad with mayonnaise pH of 3.7 than those with pH of 4.1–5.1 after a 10-day storage, the levels in all samples were 7.0 log₁₀ cfu/g after 12 days (Fig. 3c). The growth of *L. monocytogenes* in vacuum-packed salads (Fig. 3d–f) prepared with mayonnaise adjusted to pH 3.7–5.1 was similar to that in aerobic packages. The initial inoculum of *L. monocytogenes* was approximately 1.5 log₁₀ cfu/g. After a 22-day storage at 4 °C, *L. monocytogenes* levels were >6.5 log₁₀ cfu/g in salad prepared with mayonnaise pH of 4.0, 4.7, and 5.1, 6.0 log₁₀ cfu/g with pH of 4.4, and 3.5 log₁₀ cfu/g with pH of 3.7 (Fig. 3d). At 8 °C, the levels of *L. monocytogenes* were 7.0 log₁₀ cfu/g in salad with mayonnaise pH of 4.7 and 5.1 after 12 days, while the levels were 6.0 log₁₀ cfu/g in salad with mayonnaise pH of 3.7–4.4 after 16 days (Fig. 3e). At 12 °C, *L. monocytogenes* levels in all salad reached >6.5 log₁₀ cfu/g after a 9-day storage (Fig. 3f). In vacuum packaged product, salad prepared with mayonnaise pH of 3.7 and stored at 4 °C supported the slowest growth of *L. monocytogenes* (3.5 log₁₀ cfu/g after 22 days) (Fig. 3d).

The lag phase duration (LPD, h) and GR of *L. monocytogenes* in seafood salad with various mayonnaise pH levels and stored under aerobic and vacuum conditions calculated with the D-model are shown in Table 2. Under aerobic conditions, the LPD ranged from 0 h in salad with mayonnaise pH 5.1 to ~100 h in salad with mayonnaise pH 3.7. The LPD were not significantly different between mayonnaise pH of 3.7 (98 h) and 4.0 (107 h) stored at 4 °C and among mayonnaise pH of 3.7 (91 h), 4.1 (99 h), and 4.4 (76 h) stored at 8 °C. At 12 °C, the LPD was significantly greater (80 h) in salad with mayonnaise pH of 3.7 than salad with pH 4.0–5.1 (<26 h). Under vacuum conditions, the LPD in salad with mayonnaise pH 3.7 and 4.0 at 4 °C, with mayonnaise pH 3.7–4.7 at 8 °C, and with mayonnaise pH 3.7 and 4.0 at 12 °C were significantly lower ($p<0.05$) compared with otherwise similar salads stored aerobically. The

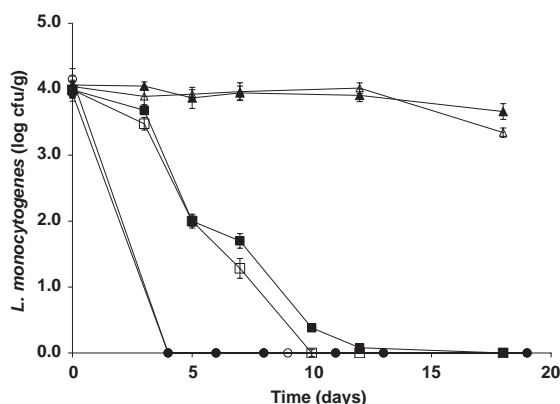


Fig. 2. Survival of *L. monocytogenes* in mayonnaise adjusted to various pH at 4 and 8 °C; pH 4.0 at 4 °C (●), pH 4.5 at 4 °C (■), pH 5.0 at 4 °C (▲), pH 4.0 at 8 °C (○), pH 4.5 at 8 °C (□), and pH 5.0 at 8 °C (△).

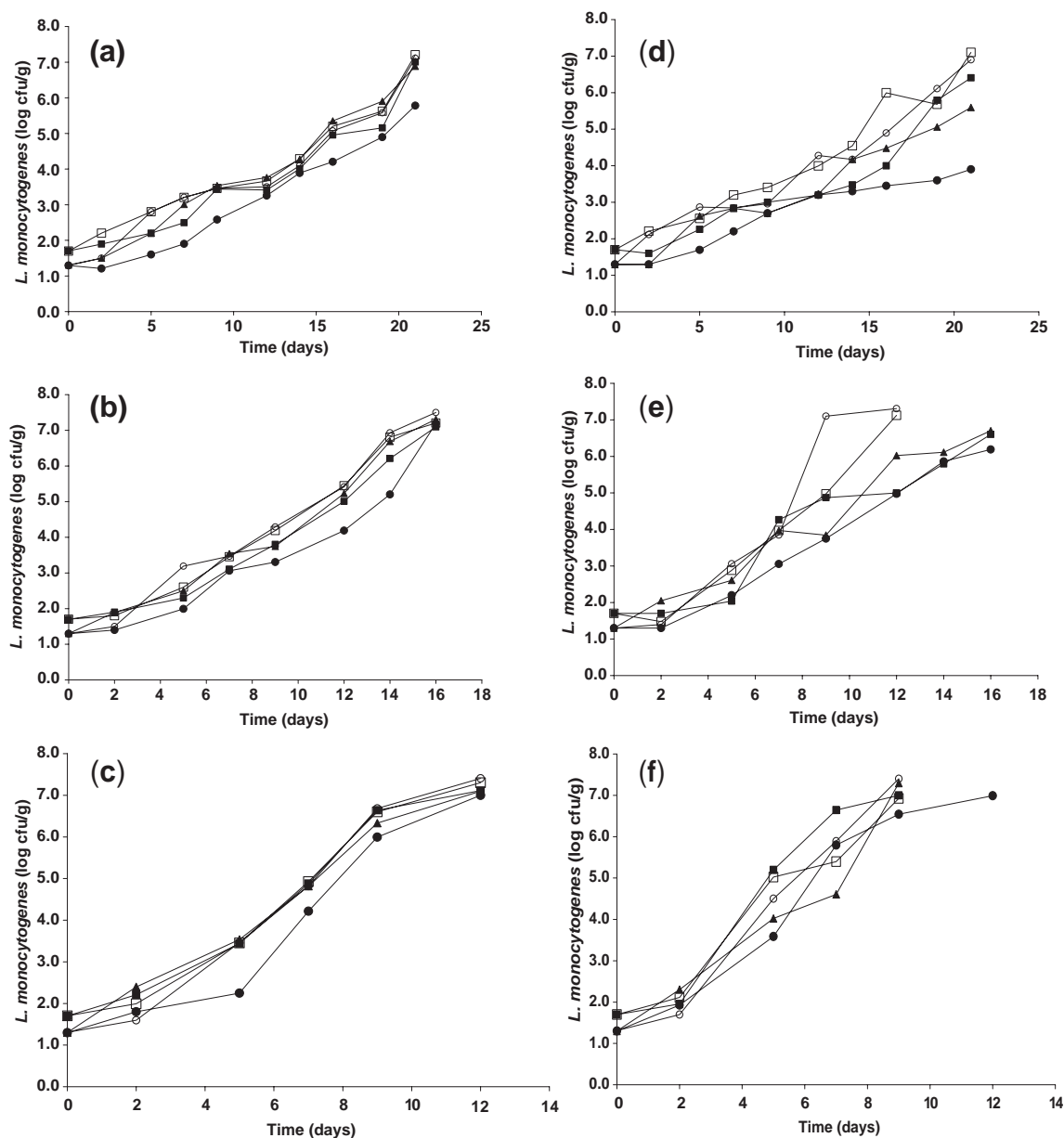


Fig. 3. Growth of *L. monocytogenes* in seafood salad with mayonnaise adjusted to pH 3.7 (●), 4.0 (■), 4.4 (▲), 4.7 (○), and 5.1 (□) stored at 4, 8, and 12 °C under aerobic and vacuum conditions; (a) 4 °C-aerobic, (b) 8 °C-aerobic, (c) 12 °C-aerobic, (d) 4 °C-vacuum, (e) 8 °C-vacuum, and (f) 12 °C-vacuum.

extension of LPD of *L. monocytogenes* by aerobic condition was also reported by Jacxsens et al. (2001) that high oxygen atmosphere extended *L. monocytogenes* LPD in RTE vegetables. Under aerobic condition at 4 and 8 °C, the GR of *L. monocytogenes* in salad with mayonnaise of pH 5.1 at 4 °C (0.0096

log₁₀ cfu/h) and 8 °C (0.0155 log₁₀ cfu/h) were significantly lower ($p < 0.05$) than those with mayonnaise pH of 3.7–4.7; however, no LPD was observed. At 12 °C, the GR in salad with mayonnaise pH of 5.1 was the lowest (0.0216 log₁₀ cfu/g), but it was only significantly different from the salad with

Table 2

Lag phase duration (LPD, h) and growth rate (GR, log cfu/h) of *L. monocytogenes* in seafood salad stored at 4, 8, and 12 °C under aerobic and vacuum conditions

Temperature (°C)	Mayonnaise pH	LPD (h) ^a		GR (log cfu/h) ^a	
		Aerobic	Vacuum	Aerobic	Vacuum
4	3.7	97.6 ^{a,A}	30.3 ^{a,A}	0.0107 ^{a,A}	0.0075 ^{a,A}
	4.0	107.2 ^{b,A}	89.4 ^{a,b,A}	0.0112 ^b	0.0101 ^{a,b}
	4.4	40.2 ^{a,b,c}	13.6 ^b	0.0113 ^{a,c,A}	0.0086 ^{a,b,c,A}
	4.7	12.3 ^{a,b}	7.3 ^b	0.0103 ^{b,c,d}	0.0102 ^{a,c}
	5.1	0.0 ^{a,b,c}	0.0 ^b	0.0096 ^{a,b,c,d}	0.0102 ^{a,c}
8	3.7	91.2 ^{a,A}	51.9 ^{a,A}	0.0175 ^{a,A}	0.0152 ^{a,A}
	4.0	99.0 ^{b,A}	32.7 ^{b,A}	0.0182 ^{b,A}	0.0137 ^{b,A}
	4.4	76.2 ^{b,c,A}	6.9 ^{a,A}	0.0185 ^{a,A}	0.0147 ^{a,A}
	4.7	34.7 ^{a,b,c,d,A}	0.0 ^{a,b,A}	0.0176 ^{d,A}	0.0244 ^{a,b,c,d,A}
	5.1	0.0 ^{a,b,c,d}	0.0 ^b	0.0155 ^{a,b,c,d,A}	0.0195 ^{a,b,c,d,A}
12	3.7	80.7 ^{a,A}	4.4 ^{a,A}	0.0283 ^{a,A}	0.0224 ^{a,A}
	4.0	25.9 ^{a,b,A}	0.0 ^{a,A}	0.0222 ^{a,A}	0.0281 ^{a,b,A}
	4.4	4.1 ^{a,b}	0.0 ^a	0.0220 ^{a,A}	0.0257 ^{a,c,A}
	4.7	0.0 ^{a,b}	0.0 ^a	0.0235 ^{a,A}	0.0298 ^{a,c,d,A}
	5.1	0.0 ^{a,b}	0.0 ^a	0.0216 ^a	0.0251 ^{a,b,d}

^{a-d} At the same temperature, means in the column with same superscript are significantly different ($p < 0.05$).

^a Means of LPD and GR from duplicate samples of each treatment.

^A At the same temperature and mayonnaise pH, means of LPD and GR, respectively, are significantly different ($p < 0.05$).

mayonnaise pH of 3.7 (0.0283 log₁₀ cfu/g). The results showed that *L. monocytogenes* had a lower GR in salad with mayonnaise of pH 5.1 than in salad with mayonnaise pH of 3.7–4.7 under aerobic conditions. At the same mayonnaise pH, the GR increased as the storage temperature increased, that is with mayonnaise pH of 3.7, the GR was 0.0107 log₁₀ cfu/h at 4 °C, 0.0175 log₁₀ cfu/h at 8 °C, and 0.0283 log₁₀ cfu/h at 12 °C. Under vacuum condition, the GR in salads with mayonnaise pH 3.7 (0.0075 log₁₀ cfu/h) and 4.4 (0.0086 log₁₀ cfu/h) were significantly lower than salad with mayonnaise pH 4.0 (0.0101 log₁₀ cfu/h), 4.7 (0.0102 log₁₀ cfu/h), and 5.1 (0.0102 log₁₀ cfu/h) stored at 4 and 8 °C. At 12 °C, the GR in salad with mayonnaise pH 3.7 (0.0224 log₁₀ cfu/h) was significantly lower than salad with mayonnaise pH 4.1–5.1 (>0.0251 log₁₀ cfu/h). As in under aerobic condition, the GR of *L. monocytogenes* in salads increased as the storage temperature increased under vacuum conditions.

L. monocytogenes was inactivated in mayonnaise with pH 4.0, 4.5, and was unable to grow in mayonnaise with pH 5.0 while stored at 4 and 8 °C. The levels of *L. monocytogenes* in seafood salad with mayonnaise pH of 4.0 to 5.1 increased 5.0 log₁₀ cfu/g after 22 days at 4 °C, 16 days at 8 °C, and 12 days at

12 °C under aerobic conditions. In seafood alone, the same increase in levels of *L. monocytogenes* were achieved after 4 days at 8 and 12 °C, and 7 days at 4 °C. Comparing the growth rates of *L. monocytogenes* in seafood, salad, and mayonnaise at 4 °C, the growth rates in seafood salad (0.0096–0.0113 log cfu/h) were between those in seafood (0.024 log₁₀ cfu/h) and mayonnaise (<–0.001 to –0.0041 log₁₀ cfu/h), indicating the inactivation/growth-inhibitory effect of mayonnaise on *L. monocytogenes* was reduced by the food components to a growth-delaying effect. A similar result was reported by Erickson and Jenkins (1991) showing *L. monocytogenes* was inactivated in regular (pH 3.9), reduced calorie (pH 3.9), and cholesterol-free (pH 3.9) mayonnaise dressing at ambient temperature (26.6 °C), but growth of *L. monocytogenes* was observed in a chicken salad (pH 5.7) prepared with commercial mayonnaise after a 7-day storage at 4 °C (Erickson et al., 1993). The critical formulation that maintains microbiological safety of commercial mayonnaise is ≥0.25% acetic acid and pH≤4.1 (Smittle, 1977). Therefore, it is reasonable to assume that food components in salads significantly buffer mayonnaise acidity (pH) and/or reduced other inhibitory factors in mayonnaise, such as a_w . In comparison to the study of Erickson et al. (1993), a

shorter growth delay (~4 days) of *L. monocytogenes* in seafood salad was observed at 4 °C in this study. This may be due to the fact that *L. monocytogenes* was inoculated onto seafood components instead of onto the finished salad. The surfaces of the seafood components provide a better growth environment and protection to *L. monocytogenes* cells. Sources of *L. monocytogenes* contamination in seafood salad most likely come from the seafood components and/or recontamination during preparation and storage of salad. The contamination of *L. monocytogenes* on seafood components before mixing with mayonnaise presents a worst case scenario as observed in this study.

3.5. Growth models

The mathematical equations describing the growth of *L. monocytogenes* in terms of LPD and GR in seafood salad as a function of storage temperature and mayonnaise pH under aerobic and aerobic conditions are as follows:

Aerobic conditions

LPD (h) = $620.83 + 2.916 * \text{temperature} - 186.5 * \text{pH} + 3.841 * \text{temperature} * \text{pH} - 1.463 * (\text{temperature})^2 + 9.879 * (\text{pH})^2$. ($p < 0.001$).

Significance: $\text{pH} > (\text{temperature})^2 > \text{temperature} * \text{pH} > (\text{pH})^2 > \text{temperature}$.

GR (\log_{10} cfu/h) = $-0.00313 + 0.00338 * \text{temperature} + 0.00214 * \text{pH} - 0.00031 * \text{temperature} * \text{pH} - 0.000024 * (\text{temperature})^2 - 0.000169 * (\text{pH})^2$. ($p < 0.001$).

Significance: $\text{temperature} > \text{temperature} * \text{pH} > (\text{temperature})^2 > \text{pH} > (\text{pH})^2$.

Vacuum conditions

LPD (h) = $429.59 - 21.45 * \text{temperature} - 115.01 * \text{pH} + 4.987 * \text{temperature} * \text{pH} - 0.237 * (\text{temperature})^2 + 5.38 * (\text{pH})^2$. ($p < 0.01$).

Significance: $\text{pH} > \text{temperature} > \text{temperature} * \text{pH} > (\text{temperature})^2 > (\text{pH})^2$.

GR (\log_{10} cfu/h) = $-0.07323 + 0.00168 * \text{temperature} + 0.03188 * \text{pH} - 0.000038 * \text{temperature} * \text{pH} + 0.000017 * (\text{temperature})^2 - 0.00333 * (\text{pH})^2$. ($p < 0.001$).

Significance: $\text{temperature} > \text{pH} > (\text{pH})^2 > (\text{temperature})^2 > \text{temperature} * \text{pH}$.

The p value for each equation indicates that both the storage temperature and mayonnaise pH significantly affected the LPD and GR of *L. monocytogenes*

in seafood salad stored at 4–12 °C. Measurements of the significance of temperature, pH, and interactions illustrate that LPD and GR are most sensitive to mayonnaise pH and storage temperature, respectively. While the interactions of temperature and/or pH were generally less significant than temperature and pH, they were included in the equations to better describe the growth of *L. monocytogenes* in seafood salad. Future research will include lower storage temperatures and lower pH of mayonnaise to improve the models utility to food industries and risk assessors.

4. Summary

This study showed that the LPD of *L. monocytogenes* in seafood salad was influenced by the mayonnaise pH, while the GR was affected by the storage temperature. In this study the slowest growth of *L. monocytogenes* in seafood salad was observed in salad prepared with a mayonnaise pH of 3.7 and stored at 4 °C. This confirms the recommended cold-holding practice that potentially hazardous foods be held at temperature ≤ 5 °C with a shelf life of ≤ 7 days (FDA, 2001). For salad dishes prepared with non-real mayonnaise (e.g., reduced calories and/or fat mayonnaise) that may have a higher pH, a shorter storage time should be considered.

Food scientists have continued to conduct studies to determine factors, e.g., pH, a_w , and additives, and their levels in foods that can effectively control growth of microorganisms in food products. Effective factors and levels reported by researchers are commonly referenced with respect to systems in which these factors and levels are evenly dispersed in the product. In a food system with multiple components, the factors and levels cannot be guaranteed to be present in all regions of the product. With a growing number of formulated RTE foods, behaviors of microorganisms in a food system with multiple components of various properties deserve further investigation.

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